

Appln. No. 10/020,923

New Attorney Docket No.: 8109.003.USDV

d.) **Amendments to the Claims.**

Please cancel claims 31 and 32, amend claims 11 and 29, and add new claims 33-36, all without prejudice or disclaimer of the subject matter thereof, as follows:

Claims 1.-5. (canceled).

Claim 6. (withdrawn) A method for detecting 10,000 cfu/ml or less of actively respiring microorganisms in a sample comprising:

trapping the microorganisms of said sample on a solid filtration membrane;

incubating the trapped microorganisms with a nutrient medium containing a predetermined amount of a viability substrate, wherein metabolism of said viability substrate by the microorganisms produces a viability marker;

digesting the microorganisms;

contacting primary antibodies prepared against a substituted formazan with the digested microorganisms to capture said primary antibodies;

contacting secondary antibodies prepared against the primary antibodies and conjugated with a detectable marker to captured primary antibodies; and

detecting the secondary antibodies that are bound to the captured primary antibodies.

Claim 7. (withdrawn) A method for detecting 10,000 cfu/ml or less of microorganisms comprising:

incubating the microorganisms with a nutrient medium containing a predetermined amount of a viability substrate, wherein metabolism of said viability substrate by the microorganisms produces a viability marker

digesting the microorganisms by incubation with a lysozyme to form a cellular debris, wherein the viability marker is adsorbed on a surface of the cellular debris;

immobilizing primary antibodies specific for the viability marker on a solid support;

contacting the digested microorganisms with the immobilized primary antibodies thereby capturing the microorganisms; and

detecting the presence of the viability marker.

Claim 8. (withdrawn) The method of claim 7 wherein the step of detecting comprises:

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contacting the captured digested microorganisms with a reporter antibody prepared from the primary antibody, the reporter antibody being conjugated to a detectable marker; and detecting the reporter antibodies that bind to the captured digested microorganisms.

Claim 9. (withdrawn) The method of claim 7 wherein the step of detecting comprises detecting the captured viability marker by detecting a change in a physical, a chemical, an optical, or an electrical property of the solid support.

Claim 10. (withdrawn) The method of claim 7 further comprising the steps of:

incubating the viability marker with a primary antibody specific for the viability marker and conjugated to a reporter molecule, thereby forming a primary antibody-antigen-reporter molecule sandwich; and

detecting the reporter molecule.

Claim 11. (currently amended) A method for detecting 10,000 cfu/ml or less of microorganisms comprising:

incubating the microorganisms with a nutrient medium containing a predetermined amount of a viability substrate, wherein metabolism of said viability substrate by the microorganisms produces a viability marker;

digesting the microorganisms;

incubating the digested microorganisms with a primary antibody specific for the viability marker;

conjugating the primary antibody to a reporter molecule to form a reporter-primary antibody complex; and

detecting reporter molecules that form reporter-primary antibody complexes; and

determining the amount of microorganisms from the reporter-primary antibody complexes detected, wherein the microorganisms are bacteria and the method is performed in less than eight hours.

Claim 12. (withdrawn) A method for detecting less than 10,000 cfu/ml of actively respiring microorganisms in a sample comprising:

incubating the actively respiring microorganisms with a nutrient medium containing a predetermined amount of a viability substrate, wherein metabolism of said viability substrate by

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the microorganisms produces a viability marker;
digesting the microorganisms;
contacting a primary antibody prepared against a substituted formazan with the digested microorganisms;
contacting a secondary antibody prepared against the primary antibody, the secondary antibody being conjugated to a reporter molecule; and
detecting the reporter molecule.

Claim 13. (withdrawn) The method of claim 12 further comprising the step of trapping the actively respiring microorganisms on a solid filtration membrane.

Claim 14. (withdrawn) The method of claim 12 wherein the reporter molecule comprises an enzyme, a bioluminescent protein, a radioisotope, a chemiluminescent dye, a visible dye, a latex particle, a magnetic particle or a fluorescent dye.

Claim 15. (withdrawn) The method of claim 12 wherein the sample is a clinical sample, a food sample, a cosmetic sample, a pharmaceutical sample, an industrial sample or an environmental sample.

Claim 16. (withdrawn) The method of claim 12 wherein the sample is a blood sample, a tissue sample, a tissue homogenate sample or a bodily fluid sample.

Claim 17. (withdrawn) The method of claim 12 wherein the microorganisms comprises a single species of microorganisms or a mixed population of microorganisms.

Claim 18. (withdrawn) The method of claim 12 wherein the sample contains less than 1,000 cfu/mL.

Claim 19. (withdrawn) The method of claim 12 wherein the detecting takes less than two hours.

Claim 20.-24. (canceled).

Claim 25. (withdrawn) The method of claim 6, wherein the sample contains less than 1,000 cfu/mL.

Claim 26. (withdrawn) The method of claim 6, which takes less than two hours.

Claim 27. (withdrawn) The method of claim 7, wherein the microorganisms comprise 1,000

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cfu/mL or less.

Claim 28. (withdrawn) The method of claim 7, which takes less than two hours.

Claim 29. (currently amended) The method of claim 11, wherein the microorganisms determined from the reporter-primary antibody complexes detected comprise 1,000 cfu/mL or less.

Claim 30. (previously presented) The method of claim 11, which takes less than two hours.

Claim 31. (canceled).

Claim 32. (canceled).

Please add the following as new claims 33-36:

Claim 33. (new) The method of claim 11 further comprising the step of trapping the actively respiring microorganisms on a solid filtration membrane.

Claim 34. (new) The method of claim 11 wherein the reporter molecule comprises an enzyme, a bioluminescent protein, a radioisotope, a chemiluminescent dye, a visible dye, a latex particle, a magnetic particle or a fluorescent dye.

Claim 35. (new) The method of claim 11 wherein the microorganisms are obtained from a clinical sample, a food sample, a cosmetic sample, a pharmaceutical sample, an industrial sample, an environmental sample, a blood sample, a tissue sample, a tissue homogenate sample or a bodily fluid sample.

Claim 36. (new) The method of claim 11 wherein the microorganisms comprises a single species of microorganism or a mixed population of microorganisms.